

***Macarostola zehntneri* (Snellen) (Lepidoptera, Gracillariidae) newly recorded from Japan and Taiwan with description of genital structures and new host plants**

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Abstract A gracillariid moth, *Macarostola zehntneri* (Snellen, 1902) is newly recorded from Japan and Taiwan. The genital structures of this species are described for the first time. New host plants and larval habit are described with photographs. DNA barcode data of this species is provided for the first time.

Key words DNA barcodes, leafminer, Myrtaceae, Okinawa, *Syzygium*.

Introduction

Macarostola zehntneri (Snellen, 1902) was described based on specimens from Java, Indonesia. Meyrick (1932) reported the biology of this species on *Eugenia jambolana* (= *Syzygium cumini*), Myrtaceae from Bombay, India. The host plants of *Macarostola* all belong to the Myrtaceae except for the following Japanese species (Kumata, 1977). In Japan, only one species, *Macarostola japonica* Kumata, 1977 (host: *Euscaphis japonica*, Staphyleaceae) has been described. Recently, De Prins and De Prins (2016) placed photos on the internet of Japanese adult specimens treated as *M. zehntneri*, which were collected on *Eugenia javanica* (= *S. samarangense*) by the third author (Kumata) in the Ishigaki Is., Okinawa Prefecture. The photos of the specimen were identified to *M. zehntneri* by the third author (Fig. 1F), but no paper has been published to record this species from Japan.

Dr Y. Yoshiyasu collected rolled leaves of *S. jambos* in Okinawajima Is., Okinawa Pref. in March, 2016. At first, he considered the leaf rolls to be nests of thyridid larva, but gracillariid larvae and serpentine mines had been observed in them and some adults of *M. zehntneri* emerged.

In the course of the identification of this species, we found specimens of this species collected in Taiwan kept in the insect collection of Osaka Prefecture University. In this paper, *Macarostola zehntneri* is newly recorded from Japan and Taiwan with a report of the larval habit on new host plants, and male and female genitalia are described for the first time. In addition, DNA barcode data of this species is provided using species identification.

Material and methods

For genital dissections, the whole abdomen was removed and boiled for 3–4 min in 10 % aqueous KOH, and residual scales and soft parts were removed in 70% ethanol. Genitalia were then stained in f acetocarmine for 1–2 h, dehydrated in a series of 70–100 % ethanol and mounted in canada balsam on a glass slide. A sample of an emerged adult was preserved in 99 % ethanol for DNA sequencing. Total DNA was extracted from middle and hind legs. Primer sets LCO 1490 (fwd) and HCO 2198 (rev) (Folmer *et al.*, 1994) were used to amplify the DNA barcode region, a 658 bp fragment of mitochondrial COI. The obtained sequence data (voucher no. SK-096) was deposited in GenBank [accession no. LC168125]. Adults specimens were preserved in the Entomological Laboratory, Osaka Prefecture University (OPU) and the Hokkaido University Museum (HUM). Scientific names of plants follow the Missouri Botanical Garden *Tropicos* database (2016).

***Macarostola zehntneri* (Snellen, 1902) (Figs 1–4)**

Pammeces zehntneri Snellen, 1902: 91, pl. 6 (6).

Parectopa zehntneri: Meyrick, 1912: 49; Meyrick, 1932: 270.

Macarostola zehntneri: Kumata, 1977: 36.

Type locality. Indonesia (Java)

Material examined 18 (8 ♂ 10 ♀).

Host *Syzygium jambos*: JAPAN: 1 ♂ 3 ♀, Nakijin, Kunigami, Okinawa Is., Okinawa Pref., 22–23.iii.2016 em., Y. Yoshiyasu leg., 1.iii.2016 (larva), genitalia slide no. OPU-SK597–600 ; INDIA: 1 ♂, Bangalore, Karnataka, 6.ii.1978 em., F. Sakagami and T. Kumata leg., host: *Eugenia jambos* (= *S. jambos*) breeding no. Ind-12, deposited in HUM.

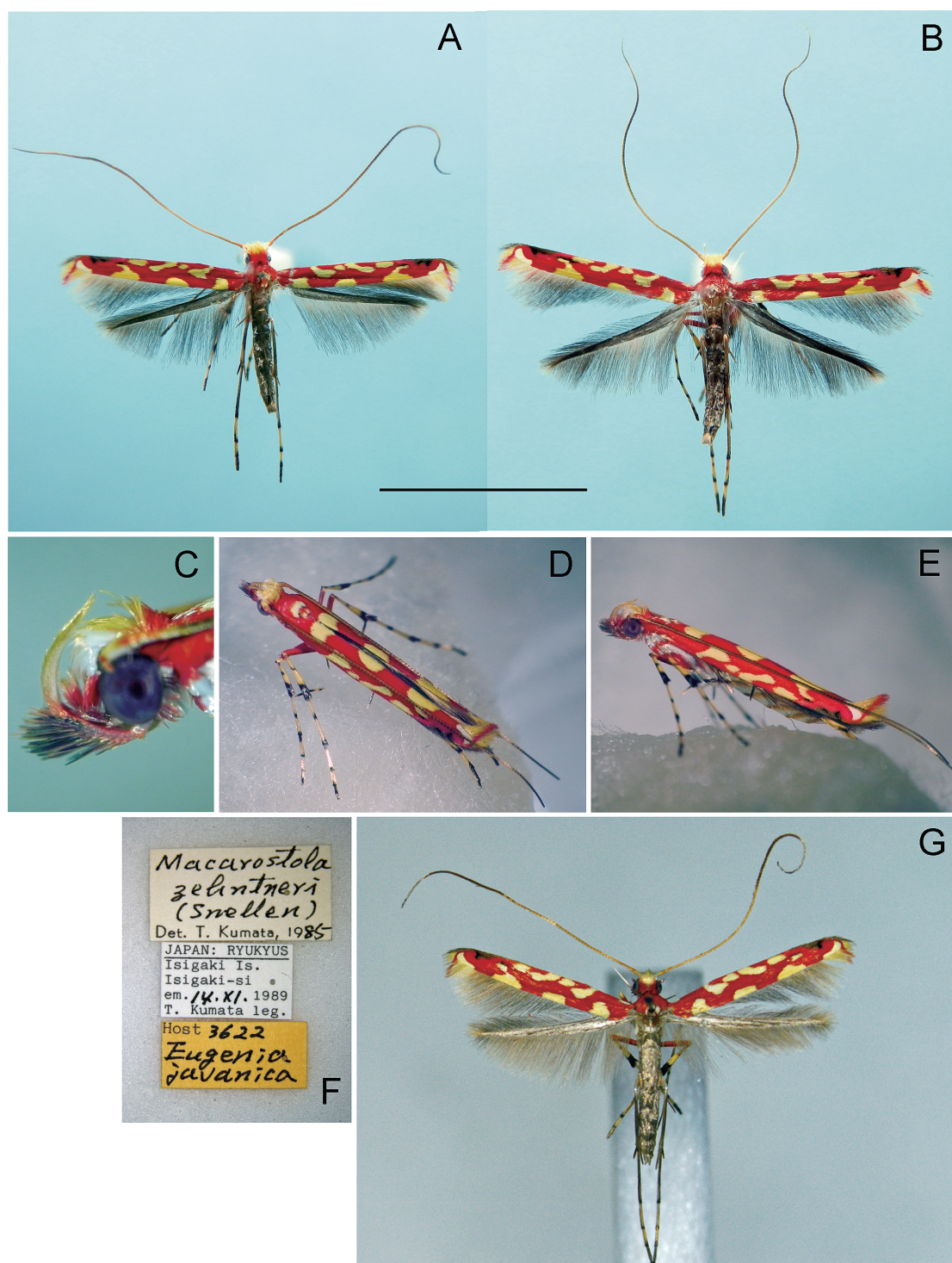


Fig. 1. Adults of *Macarostola zehntneri* (Snellen, 1902), Okinawa Pref. A–E host *Syzygium jambos*; F, G *S. samarangense*. A: Male. B: Female. C: Head, lateral view. D: Resting posture of the adult, dorsal view. E: Same, lateral view. F: Labels of G. G: Adult, photo taken by Dr J. De Prins. Scale bar: 5 mm.

Host *Eugenia javanica* (= *S. samarangense*): JAPAN: 6 ♂ 5 ♀, "Ishigaki-si, Ishigaki Is., Okinawa Pref., 6–28.xi.1989 em., T. Kumata leg., *Macarostola zehntneri* (Snellen) Det. T. Kumata" in HUM.

Host "Renmu" (= *S. samarangense*): TAIWAN: 1 ♀, Kagi, 13.iv.1935 em., S. Issiki leg., 23.iii.1935 (larva), SK601; 1 ♀, Tainan-Hsien, Kuantzuling, 30.vi.1974 em., H. Kuroko leg., 20.vi.1974 (larva), SK602.

Diagnosis. This species has a white to yellow head and seven or eight yellowish and two blackish blotches in the forewing; the tornal one is white and L-shaped, and the three or four costal streaks and three dorsal blotches are whitish yellow in coloration. Another Japanese species, *M. japonica* Kumata is distinguished from this species by the crimson red head, the three white costal streaks and the absence of blackish scales in the forewing, the more obovate valva in the male genitalia, and the short needle-shaped signa in the female genitalia.

Additional description. Adult. (Fig. 1) Wing expanse 8.1–10.3 mm; forewing 3.1–4.9 mm. The type specimens have three costal streaks in the forewing (Snellen, 1902, pl. 6-fig. 6a), while the Japanese specimens have three or four costal streaks: probably the 1st streak is sometimes divided into two streaks (Fig. 1A). The male 8th abdominal segment has coremata, 1/2 the length of the valva (Fig. 2B) and a tongue-shaped projection on the posterior part of the tergite, with a pair of processes from the projection reaching towards its anterior part (Fig. 2C).

Male genitalia. (Fig. 2A, B) (1 preparation examined) Tegumen weakly sclerotized with three fine setae on either side. Valva nearly obovate in shape, narrowing basally, densely covered with short setae on distal 1/2 of inner surface and some very long setae on outer surface. Vinculum very narrow, with a very slender saccus about 4/5 length of valva. Phallus straight, 1.8–2.0 x length of valva, anterior part slightly widening; membrane-enclosed part of apical 1/5 (=vesica: Kumata, 1977) covered with numerous microscopic thorns.

Female genitalia. (Fig. 2D, E) (5 preparations examined) Ostium bursae weakly sclerotized; sterigma around ostium bursae membranous and smooth. Antrum undeveloped. Ductus bursae long, tubular, slightly widened towards corpus bursae. Corpus bursae oblong with two spatular-shaped signa, which are covered with acute spines, the long one about twice length of short one.

Distribution. Japan (Okinawa Prefecture); Taiwan; Indonesia (Snellen, 1902); India (Meyrick, 1932). New to Japan and Taiwan.

Host plants. *Syzygium cumini* (L.) Skeels (Meyrick, 1932) in India; *S. jambos* (L.) Alston in India and Japan; *S.*

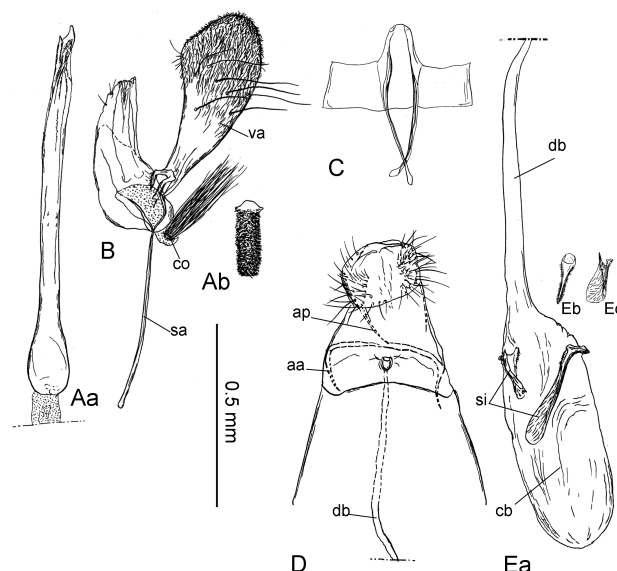


Fig. 2. Genitalia of *Macarostola zehntneri*. A–C Male. D–E Female. Aa: Phallus, ventral view. Ab: Microscopic thorns of vesica. B: Whole genitalia with left valva removed, ventral view. C: Abdominal segment VIII of male, dorsal view. D: Whole genitalia except corpus bursae, ventral view. Ea: Corpus bursae and ductus bursae, ventral view. Eb: Short signum, lateral view. Ec: Same, dorsal view. Abbreviations: aa: apophysis anterioris; ap: apophysis posterioris; cb: corpus bursae; co: coremata; db: ductus bursae; ob: ostium bursae; sa: saccus; si: signa; va: valva; ve: vecica; vi: vinculum.

samarangense (Blume) Merr. and L.M.Perry in Taiwan and Japan, Myrtaceae.

Biology. (Fig. 3) In the present study, we observed the larva of this species mining leaves of *S. jambos* in Japan. The young larva mines the abaxial side of the leaf, forming a linear serpentine mine, about ~7 cm in length, 0.5 mm in width and white in coloration. The later larva, probably the 4th instar, leaves the mine and transfers to the tip of the leaf; it cuts a semicircular shape from the middle of the leaf edge towards the apex on large leaves (Fig. 3C), or on small leaves rolls without cutting (Fig. 3G–K); the leaf or cut leaf tissue is rolled to form a cone on the abaxial side of the leaf; cones are 10–15 mm in length and 5–10 mm in width. The larva continues to feed inside the cone. When full-grown the larva leaves the cone to pupate. Final instar larvae spun a white cocoon at the leaf margin, which was strongly curled by contraction of the cocoon silk (Fig. 3C–E); the cocoon is narrow and long, 10–20 mm in length.

Remarks. De Prins and De Prins (2016) placed a photo of a Japanese specimen treated as *Macarostola zehntneri* on the internet. Although the genitalia of this specimen was not examined, we regard this specimen as conspecific with *M. zehntneri* because of the similar forewing pattern (Fig. 1G, same images: De Prins and De Prins, 2016, <http://www.gracillariidae>).

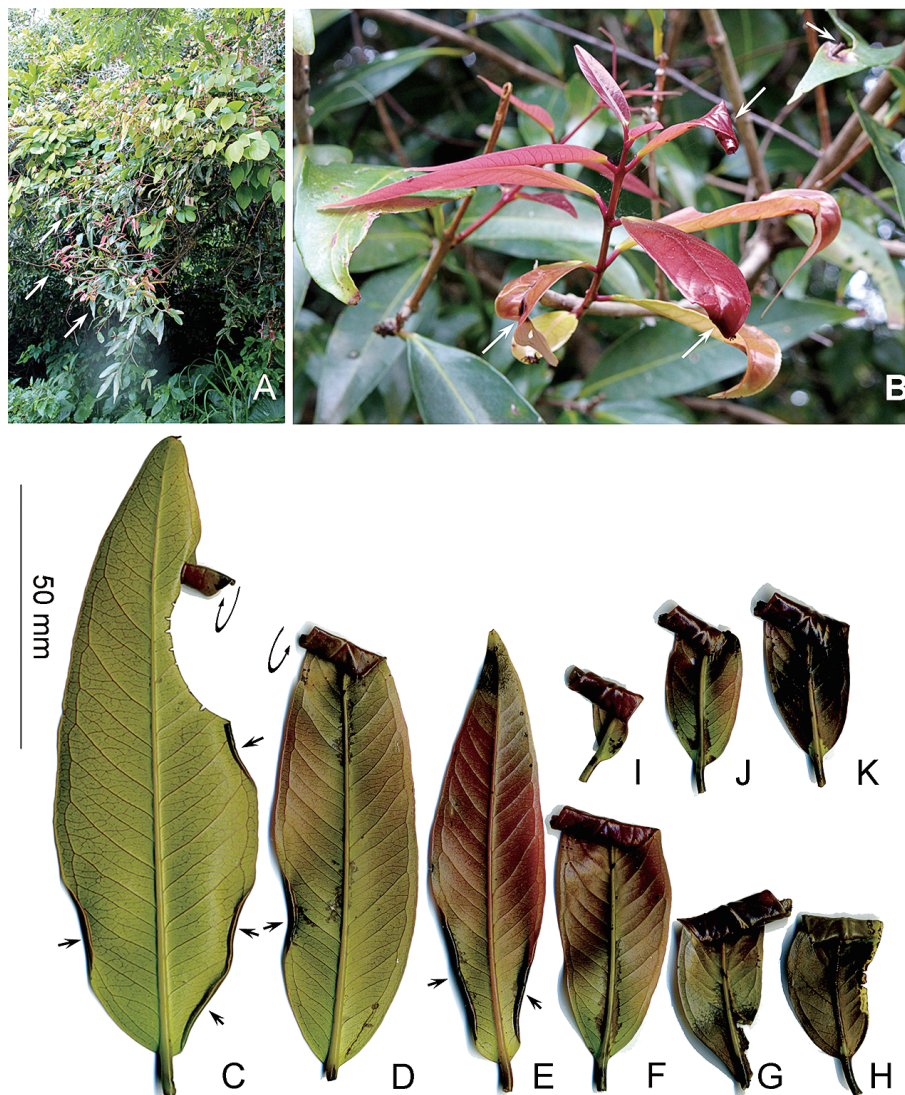


Fig. 3. Mines of *Macarostola zehntneri* and the host plants *Syzygium jambos*. A: Habitat and hostplant trees. B: Rolled mines. C-E: Cocoons and rolled mines. F: Rolled mines and small leaves. White arrows show host plants (A) and leaf mines (B); Black arrows show cocoons.

net/species/show/1866). *S. samarangense* is newly recorded as a host plant of *M. zehntneri*. The host plants of this species have been introduced to cultivation in Japan and Taiwan and escaped into the wild. It is unknown whether the larva utilized native species of Myrtaceae in Japan and Taiwan. Although the larval habits of this species are similar to those of other congeners, e.g. *M. pontificalis* (Meyrick) (Clarke, 1971) and *M. japonica* (Kumata, 1977), *M. zehntneri* is distinguished by its host plants and the boat-shaped cocoon on the leaf surface.

The COI DNA barcoding region was sequenced using a Japanese specimen. We performed sequence comparison to check species independency for *M. zehntneri* by using the BOLD Identification System (IDS) from the BOLD website ([\[barcodeoflife.org/\]\(http://barcodeoflife.org/\)\) \[accessed 1 July 2016\]. The sequence of *M. zehntneri* \(Fig. 4, sample ID: SK-96\) was clearly distinguished from all other *Macarostola* species registered in BOLD \(including *M. japonica* and the Australian species, *M. ida* \(Meyrick\)\) with more than 6% differences \(Fig. 4\). The nearest neighbor of *M. zehntneri* in the BOLD database was an unidentified Australian species \(Fig. 4\).](http://www.</p>
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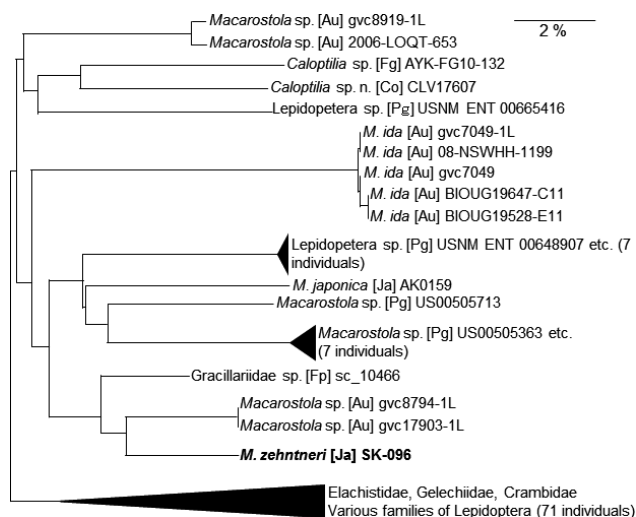


Fig. 4. A tree estimated by neighbor-joining analysis under the Kimura's two-parameter distance model using DNA barcode sequence, retrieved from BOLD identification system (IDS). Branch lengths are proportional to distances. Sequence with ID started from 'SK-' is obtained from the present study, and the others are from the BOLD database. The letters within square brackets refer to the collection site of specimens as follows: Au Australia; Co Democratic Republic of the Congo; Fg French Guiana; Fp French Polynesia; Ja Japan; Pg Papua New Guinea.

Prins (Royal Belgian Institute of Natural Sciences, Belgium) provided us with photographs of *M. zehntneri*, to whom we are deeply grateful. We also specially thank Ass. Prof N. Hirai and Dr S. Ueda (Entomological Laboratory, OPU) for their kind guidance and suggestions.

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摘要

Macarostola zehntneri (鱗翅目, ホソガ科) の日本および台湾からの新記録ならびに交尾器の記載と新寄主植物(小林茂樹・松岡 悠・久万田敏夫)

Macarostola 属は、インド～オーストラリア区から 26 種が知られ、成虫の前翅は鮮やかな橙赤色の地に白や黄色の斑紋をもち、美麗種を多く含む。幼虫は日本産種を除き、フトモモ科の植物を利用する。日本では、ベニホソガ *M. japonica* Kumata, 1977(寄主植物: ゴンズイ, ミツバウツギ科) の 1 種のみが知られていた。しかし, De Prins and De Prins (2016) は、ウェブサイト上にレンプ(フトモモ科)から得られた日本産本属の標本写真を本属の一種 *M. zehntneri* (Snellen, 1902) として掲載した。吉安は、2016 年 3 月に沖縄島において野生化したフトモモ(フトモモ科)からベニホソガ属の幼虫を採集した。羽化した成虫と大阪府立大学所蔵標本を検討した結果、前翅の斑紋の特徴から *M. zehntneri* と同定した。雌雄交尾器を初めて図示し、分布と寄主を追加するとともに、これまで報告のなかった幼虫の潜孔、マユの写真を図示した。幼虫は初め、葉にナメクジの這ったような細い潜孔を作り、その後潜孔を脱出し、葉を円錐形に巻き内部を摂食する。老熟すると巻いた葉から脱出して葉縁を強く折り曲げて細長いマユを紡いで蛹室を作り、その中で蛹化した。また、ミトコンドリア DNA の COI 領域の一部(DNA バーコード領域)の配列(658 bp)を決定し、遺伝距離を比較した結果、同属の他種(*M. japonica*, *M. ida*)と明確に区別でき、最も近かったのはオーストラリアの学名未決定種であった。

フトモモベニホソガ *Macarostola zehntneri* (Snellen, 1902) (和名新称) (Figs 1-4)

開張 8-10 mm. 前翅の斑紋は、翅頂の 2 つの黒色斑紋、後角部に L 字形の白色斑紋を除き、6 ないしは 7 つの黄色の斑紋をもつ。原記載では前縁の黄色斑紋は 3 つであるが、日本産ではしばしば第一斑紋が 2 つに分かれる。日本産のベニホソガ *M. japonica* は、頭部が橙赤色、黒色斑紋を持たない、前縁の斑紋が白色、雄交尾器のバルバはより丸みを帯びる、雌交尾器の 1 対のシグナは短く、同じ長さであるなどの点で本種と識別できる。幼虫は、両種とも葉を円錐形に巻くが、ベニホソガでは、ミツバウツギ科のゴンズイを利用し、葉縁を折らないでボート形のマユを葉上に作る。

寄主植物: フトモモ, レンプ(フトモモ科), 国外ではフト

モモ(新記録), レンブ, 同属の *Syzygium cumini* が知られる. 湾(新記録), インドネシア, インド.

分布: 日本(新記録): 沖縄(沖縄島, 石垣島); 国外では台 (Received September 15, 2016. Accepted November 2, 2016)